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THE ASSESSMENT OF ESSENTIAL DERIVATION IN GRAPEVINE

*Document prepared by experts from Spain*

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SUMMARY

1. Grapevine is a woody plant, and varieties are vegetatively propagated. For these reasons, essentially derived varieties (EDVs) normally arise from somatic mutations, and, using microsatellites, there is no difference between an initial variety and this type of EDV. Based on previous studies, with a system of 9 microsatellites thoroughly used in grapevine, it could be established that 2 different alleles (or 3, following a more conservative approach) are enough to disregard a variety as an EDV. If distinctness has been established between two varieties and no differences could be found using microsatellites, the conclusion is that one is an EDV of the other. In the case of 1 different allele (or 2), the proposal is to analyze additional microsatellites.

2. An example of the analysis of two grapevine EDVs for berry color, based on the knowledge of the molecular basis for the variation in this trait is shown, and the implications are discussed.

INTRODUCTION

3. Grapevine is one of the oldest cultures in the world. The plants are woody, and asexually multiplied through cuttings. There are a lot of varieties in the world (between 5,000 and 10,000) and many of them have been cultured over several centuries. Most are local varieties, and there are numerous synonyms (one variety having different names) and homonyms (different varieties having the same name) within and between countries.

4. There are two clearly separate markets for grapevine: wine and table. The first one is much more important economically, and stable, regarding the varieties used, because in many cases the wine producers of a given location must use certain varieties to obtain a “quality” label. So, breeding efforts are mainly focused on clone selection. The table market is much more dynamic and most of the varieties being cultivated now are different from those cultivated 30 years ago. Crossbreeding is much more active for table grapes, and new interesting varieties are quickly spread to other producer countries. As a consequence, the issue of breeders’ rights in grapevines relates mainly to these table varieties.

5. In the grapevine species, a new variety usually comes from a sexual cross, where an embryo is produced, or from an established variety through a somatic mutation and asexual propagation. In the latter case, the new variety is called a ‘sport’. If the initial variety has been granted breeders’ rights, then the sport would be an Essentially-Derived Variety (EDV), within a legal scope. Different types of somatic variants have been found in grapevine,

affecting traits such as berry color, berry taste, bunch density, formation of seeds, leaf shape, or density of hairs in the leaves. In the case of berry color, the molecular basis of the variation has already been described (Kobayashi et al. 2004): white varieties have, in a homozygous state, a retrotransposon inserted into the VvmybA1 gene that inactivates it, while colored varieties have at least one copy of this gene without the retrotransposon inserted. This seems to be responsible of most of the variation found in this trait (Lijavetzky et al. 2006).

6. These somatic variants, in addition to the natural phenotypic variation found among (and within) individual plants within a variety, and the already mentioned existence of synonyms and homonyms, have made the identification of grapevine varieties through morphology a difficult matter. A new challenge has been recently added: the assessment of essentially derived varieties. Today, the use of molecular markers is contributing to improve the identification system. Competent organizations, particularly the International Organization of Vine and Wine (OIV), Bioversity International, and UPOV have been working on systems, which are based mostly (or exclusively) on morphology. OIV has recently incorporated in its descriptor list the use of 6 DNA markers (microsatellites) studied during the GENRES 081 project (This et al. 2004) for the purpose of variety identification (OIV 2007).

7. The aim of this paper is to evaluate a system of 9 microsatellites, described in previous BMT meetings (documents BMT/9/11 “A microsatellite-based system for the protection of grapevine varieties” and BMT/10/13 “A microsatellite-based system for the identification and legal protection of grapevine varieties”) for the identification of Essentially Derived Varieties, as well as the use of the molecular basis of the variation for the berry color to characterize two different EDVs for that trait.

System of 9 microsatellites

8. Microsatellite markers were first described in grapevine in 1993 (Thomas and Scott 1993) and have been broadly used all over the world to characterize many different varieties. Many groups have developed their own markers, but the main international attempt to harmonize a microsatellite-based system for the identification of grapevine varieties was taken within the frame of the GENRES project, in Europe, and involved 10 different laboratories, including ours (This et al. 2004). Unfortunately, at the beginning of that project there was no information of map position of the microsatellites, and among the 6 markers chosen, two pairs were genetically linked. For that reason, and because they are not able to discriminate all the different varieties (not sports) tested (Martín et al. 2003), this set of 6 microsatellite is not an optimal selection. We selected a new set, based on the previously used microsatellites, and started by increasing the number of markers to 9. The criteria taken into account to make the new selection were: public availability, map position (genetically independent), high level of polymorphism, size of alleles (to allow multiplexing), and ‘quality’, according to ease of amplification, absence of known null alleles, absence of alleles differing only in 1 bp.

9. Finally, the following 9 microsatellite markers were chosen: VVS2 (Thomas and Scott 1993), VVMD5, VVMD27, VVMD28 (Bowers et al. 1996; Bowers et al. 1999), ssrVrZAG29, ssrVrZAG62, ssrVrZAG67, ssrVrZAG83 and ssrVrZAG112 (Sefc et al. 1999). A system was optimized, including the design of a multiplex PCR with the 9 markers, and capillary electrophoresis and fluorescence analysis in an automatic sequencer. Simplex PCRs were done when necessary.

10. Approximately 6,000 plants were studied using this system, mainly within two different projects: one to characterize the collection of grapevine varieties of “El Encín”, in Madrid, and the other to study the uniformity and stability of these markers using 19 varieties (Ibáñez et al. 2006).

11. The point to be considered is if there is a clear border between varieties obtained through sexual reproduction. We determined:

a) the higher number of different alleles within a variety (including sports)

b) the lower number of different alleles between different varieties (except sports)

12. From the reported study of a large grapevine collection, using 996 accessions, and considering the 9 microsatellites used here, the largest number of different alleles found was one. Regarding the second point, and from the study of 495 different varieties, two cases were found where the number of different alleles was 2. As a consequence, the theoretical minimum distance could be established as 2 alleles for these microsatellites in grapevine.

Assessment of essential derivation in grapevine

13. As explained above, grapevine is a woody plant, with a juvenile period, and so, the production of an EDV through repeated backcrossing is highly improbable. The usual methods for producing an EDV are the selection of natural (or induced) mutants, and, in the near future, genetic transformation. In any case, regarding microsatellite analysis, EDVs will very probably have an identical genotype to the initial varieties.

14. The assessment of EDV by means of morphology tests genetic conformity of the EDV with respect to the initial variety. In view of the results presented, the system of 9 microsatellite markers can easily be used for examining essential derivation. If the nine microsatellites are analyzed in two different plants, and they fully match, the conclusion will be that they are of the same variety, or one is a sport of the other. This is the situation we have found in 500 out of 501 cases during the analysis of 996 grapevine accessions.

15. If one of the concerned varieties has been granted with breeders’ rights, then the conclusion will be that it is the same variety or an EDV. The decision of which of the cases is the right one will rely on the assessment of Distinctness, using the approved descriptors. In other terms, given that an EDV has to be, by definition, a distinct variety, if a new variety has been proved to be distinct from a legally protected variety and these nine microsatellites show no differences between the varieties, then a reversal of the burden of proof should occur, and the breeder of the new variety should demonstrate that this new variety is not an EDV. In the absence of other proofs, the final conclusion should be that it is an essentially derived variety.

16. There could be other cases, where 1 or more different alleles were found between the two varieties under study. We only found one case where 1 different allele exists. The two varieties concerned were studied with 16 additional microsatellites, and no differences were found between them. These two varieties are considered distinct varieties in literature; consequently, one was considered as essentially derived from the other. Other similar cases, with 1 different allele found in plants from the same variety or sports, have been reported in the literature (Sanchez-Escribano et al. 1999; Regner et al. 2000). So, in case of 1 different allele, the suggested way to proceed is to analyze more microsatellites. If more differences are found, then the varieties should be discarded as essentially derived.

17. We found two cases where a pair of varieties differed in 2 alleles. The analysis of 16 additional microsatellites demonstrated that they were different varieties as, in both cases, they showed different genotypes in 7 of the additional microsatellites. So, the 2 different alleles corresponded to an inter-varietal difference. Based on these results, in the case of finding two or more different alleles, the conclusion would be that the two varieties arose from different embryos, and unless other evidence exists, none should be considered as essentially derived varieties.

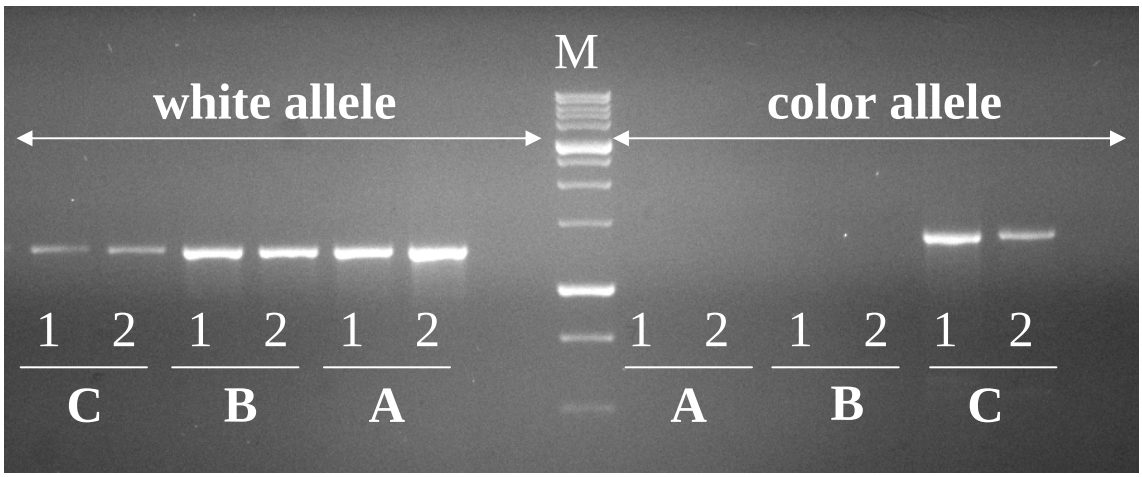
18. In practice it would be convenient to analyze more microsatellites when finding only two different alleles, and apply the direct exclusion for an essential derivation from a 3 allele difference.

A case of essential derivation and distinctness for berry color

19. Variety A, granted breeders' rights, produces green-yellow berries (white), while varieties B and C produce pink berries (rose). Plants from these 3 varieties were studied with the 9 microsatellite markers and they fully matched. Following the rules described above, the conclusion was that B and C were essentially derived from A. The pending question was if B and C were the same variety, different varieties, or distinct varieties.

20. The knowledge of the molecular basis for berry color variation in grapevine allowed us to go further in the study of the two EDVs. As explained in the Introduction section, the insertion or excision of a retrotransposon in the VvmybA1 gene seems to be responsible of most of the variation found in this trait. The insertion of the element in a homozygous state causes absence of color, while the excision of at least one of the copies of the element normally results in the presence of color. Two pairs of primers were used as described in (Lijavetzky et al. 2006), one able to amplify the allele with the insertion ('white allele'), and the other able to amplify the allele without the insertion ('color allele') in many color varieties. As grapevine is a diploid species, the hypothesis was that in one of the two alleles, originally with the insertion, an excision occurred that made the VvmybA1 active and color produced. The results obtained are shown in Figure 1. The 'white allele' was amplified in the three varieties, as expected, and the 'color allele' was not amplified in the initial, white variety A, as expected. Nevertheless, the 'color allele' did not amplify in the rose variety B, while it amplified in the other rose variety C. So, two independent mutational events, two independent excision events probably occurred in these two EDVs. In the case of variety B, the excision probably included the part of the gene where one of the primers should anneal, thus preventing amplification.

*Figure 1. Amplification of the 'white allele' and the 'color allele' in an initial variety (A) and 2 EDVs (B and C).*




21. In conclusion, there were no doubts about the fact that B and C were EDVs from A, and so its breeder had rights on them. Also, B and C are different varieties, as the gaining of the berry color occurred through independent events, but are they distinct?

22. Even though this test for the color allele could be considered a sort of an option 1 approach, as it is directly related with a specific trait, the answer for the previous question only can be achieved through a morphology study. The color of the berries is different in the two varieties, as well as the uniformity of the color. As a consequence, the final conclusion is that the two varieties are not only different, but distinct.

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